APPENDIX C

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In Re Application of:
Jorj Terry ULRICH et al.

Confirmation No. 5731

Serial No. 09/402,273

Group Art Unit 1644

Filing Date: December 13, 1999

Examiner Phuong N. HUNYH

Title: ALLERGEN FORMULATION

DECLARATION OF ALAN WORLAND WHEELER UNDER 37 C.F.R. 1.131

I, Alan Worland Wheeler, declare:

1. I am an inventor of the instant United States patent application identified by Serial No. 09/402,273 (hereinafter referred to as the "instant application").

2. The instant application was filed on December 13, 1999, as a national phase United States application from international application WO 98/44947, which was published on October 15, 1998; filed on April 3, 1998; and claims priority to GB 9706957.9 filed on April 5, 1997.

3. I understand that the international application WO 96/34626, published on November 7, 1996; filed on April 25, 1996; and of which I am also an inventor, is cited against the instant application as prior art.

4. As indicated on the first pages of WO 98/44947 and WO 96/34626, both of these international applications were owned by SmithKline Beecham PLC of Middlesex, England ("SmithKline Beecham") on their respective filing dates of April 3, 1998, and April 25, 1996.

5. Although the instant application is presently owned by Allergy Therapeutics Ltd. ("Allergy Therapeutics"), when I invented the subject matter of the instant application, I was employed at SmithKline Beecham.

6. I was employed at SmithKline Beecham as a research scientist in the area of Allergy & Immunology from 1959, to Autumn 1996 (First employed at Beecham before merge

7. The subject matter of the instant application was invented during the time of my employ at SmithKline Beecham on a date prior to the November 7, 1996, international filing date of WO 96/34626.

8. The letter attached as Exhibit A is a letter that I wrote to Dr. Ulrich prior to November 7, 1996, enclosing the draft application that would be filed in the United Kingdom on April 5, 1997, and assigned patent application number GB 9706957.9.

9. The foregoing demonstrates that the date of the invention of the instant application (see ¶ 8 above) was prior to the November 7, 1996, publication date of WO 96/34626 and that at the time of the invention of the instant application, SmithKline Beecham was the owner of the inventions described in both WO 96/34626 and WO 98/44947.

10. On June 26, 1998, as a result of an assignment agreement between SmithKline Beecham and Allergy Therapeutics, SmithKline Beecham assigned part of its allergy vaccine patent portfolio to Allergy Therapeutics; the patent portfolio assigned to Allergy Therapeutics included WO 98/44947, the priority document to the instant application.

11. Because Allergy Therapeutics was the owner of WO 98/44947 when it was filed in the United States, the instant application is owned by Allergy Therapeutics and not SmithKline Beecham.

12. All statements made herein of my own knowledge are true and all statements made herein on information and belief are believed to be true; further, all statements made herein were made with the full knowledge that willful false statements are punishable by fine, imprisonment, or both under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of the instant application or any patent issuing thereon.

Dated: 21st August 2003

Alan Worland Wheeler

SmithKline Beecham
Pharmaceuticals

FACSIMILE

To: Dr. Terry Uhlrich
RIBI IMMUNOCHEM

From: Dr Alan Wheeler
Bencard (SB)

Copy: Ronald H. Kullich
RIBIIMMUNOCHEM

Date: Time: 11:26

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Message:

Dear Terry

I have attached a draft for the proposed patent application relating to the use of MPL with tyrosine adsorbed allergens which has been prepared by our patent officer. Please could you review the application and let me have a detailed description of how the particular form of MPL used in the experiment was prepared. If there are also any pending applications or literature references that describe how the MPL was prepared, please can you let me have the detail.

The information is required so that we can both file the application and assess the inventorship.

Many thanks in advance for this information.

I am sure that you are in contact with Derek Richardson who is informing you about progress of the programme.

Regards

A.W.Wheeler

Yew Tree Bottom Road, Epsom, Surrey, KT18 5XQ.
Telephone: 01737 364000 International: +441737 364000 Fax: 01737 364100 International: +441737 364100
Telex: 8814695

Formulation

This invention relates to novel formulations for use in desensitisation therapy of allergy sufferers.

It is known that desensitisation therapy results in a changed immunological response specific for the allergens administered. Such changes are considered to be responsible for the beneficial effects of the treatment and amelioration of the symptoms of allergy.

The immunological changes responsible for benefit are not entirely understood. Although a raised allergen specific IgG antibody response is considered to be a desirable outcome of therapy, it is now believed that certain changes in the allergen specific T cell (T lymphocyte) response are more important.

Two subclasses of T cell, TH1-like and TH2-like interact with one another via various messenger molecules. In an allergic subject it appears that there is a greater allergen specific TH2 than a TH1 activity. This can lead to a high allergen specific IgE antibody level and greater eosinophil activity. These are two important components of the allergic syndrome.

A change in the above situation to one where there is greater allergen specific TH1 rather than TH2 activity is thought to be an important component of immunotherapy leading to a clinical benefit.

GB-A-1 377 074 describes a process for preparing coprecipitates of tyrosine having an allergen dispersed therein.

GB-A-1 492 973 describes a process for preparing coprecipitates of tyrosine having a modified allergen dispersed therein. The allergen has been modified by treatment with an agent, such as glutaraldehyde, which causes intra-molecular cross-linking and reduces the allergenicity of the product relative to the unmodified allergen.

3 De-O-acylated monophosphoryl lipid A (hereinafter 3-DMPL) is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/116556. 3-DMPL is an example of a substance that can enhance the TH1 over TH2 directing properties of administered allergens.

According to the present invention there is provided a pharmaceutical composition comprising tyrosine, an allergen, and 3-DMPL. Typically, the allergen is coated with and /or adsorbed onto tyrosine, for example by co-precipitation, or by simple admixing

The 3-DMPL can be mixed with the other components of the composition prior to administration. Alternatively it can be formulated together with the other components during manufacture of the product. Alternatively, it can be administered at a different site or time

than the other components. Administration can be by a number of routes including parenteral and enteral.

The allergen may be derived from any allergy causing substance, such as a pollen (e.g. ragweed or birch pollen), food, insect venom, mould, animal fur, or house dust mite (*D. farinae* or *D. pteronyssinus*). As used herein, "allergen" includes a mixture of allergens which may be from a single source or more than one source. The term "allergen" also includes peptides containing one or more epitopes of an allergen, such as allergen fragments, prepared by total synthesis, by enzymatic degradation of allergens, or by other means.

The allergen is optionally modified by reaction with a cross-linking agent such as a dialdehyde, more particularly glutaraldehyde.

A further aspect of the invention provides a process for the preparation of a pharmaceutical composition in accordance with the invention, which process comprises (a) (optionally) modifying an allergen by reaction with a cross-linking agent, (b) mixing an aqueous solution of the optionally modified allergen with a solution of tyrosine in a strong aqueous acid, (c) neutralising the mixture of solutions, thereby co-precipitating tyrosine and modified allergen, (d) mixing the product with 3-DMPL, and (e) optionally, adding a physiologically acceptable carrier.

Suitable physiologically acceptable carriers include phenol-saline and sterile water.

Typically, the allergen is modified by treatment with a dialdehyde such as glutaraldehyde, in aqueous solution at a pH of ?, typically ? and a temperature of between 0 and 100 °C, typically between 4 and 37 °C, for up to 10 hours, for example about two hours at room temperature. The ratio of allergen to glutaraldehyde is typically in the range ?, for example about ?

The product can be freeze dried or used directly in the next stage.

A solution of the modified allergen at pH 7±1, obtained either as the reaction mixture from the cross-linking process or from the solvation of a solid, is then mixed with a solution of tyrosine in a strong aqueous acid. The strong acid is usually an inorganic acid, preferably hydrochloric acid. The solution of allergen used in this step typically contains between 0.1 µg/ml and 1000 µg/ml allergen protein. The ratio of allergen: tyrosine in the mixture is typically in the range 1:4 x 10^5 to 1:4 x 10^2 w/w.

The resulting mixture of solutions of allergen and tyrosine is neutralised. By neutralisation is meant an adjustment of pH to a value within the range 4.0 to 7.5. It is important that, at no time, or at least at no prolonged time, during the neutralisation does the pH of the solution rise appreciably above 7.5. This condition can be met by vigorous stirring of the solution and by the use only of the required amount of base, if desired. Various buffering agents can usefully be added to the solutions of allergen to assist in pH control during the mixing and neutralising stages.

A particularly useful method of carrying out the neutralisation is for separate streams of the solution of tyrosine in acid and the neutralising base to be run into the solution of allergen. The rates of flow of the added solutions are controlled by pH-state, that is by equipment which regulates the flow of one or both of the solutions so that the pH of the reaction mixture remains substantially constant at a predetermined level. We have found that optimum results are usually obtained by pH control within the range 6.5 to 7.5 though the precise pH may vary according to the nature of the allergen.

The result of the neutralisation is the immediate precipitation of the tyrosine, within and/or upon which the solution of allergen is occluded and/or adsorbed. After the precipitation the mixture is either washed immediately or allowed to stand for a period of from a few hours to a day or two prior to washing. ~~Desirably the precipitate is obtained as fine as possible and this is achieved by rapid neutralisation of the solution coupled with vigorous agitation while this is being carried out.~~

The resulting precipitate may be removed from the solution by centrifugation or filtration and washed, e.g. with phenol-saline, before being resuspended in a physiologically-acceptable carrier such as phenol-saline, or sterile water, to produce an injectable composition suitable for use in desensitisation therapy in combination with 3-DMPL.

The following Example illustrates the present invention:

Example

A neutral solution of approximately 0.5 mg/ml grass pollen extract which had been partially purified by dialysis or fractionation was chemically modified by the addition of an equal volume of 0.25% w/v glutaraldehyde and the mixture stirred for approximately 2 hours at room temperature. To the above mixture was added phosphate buffer solution at a pH of 7 ±1. The allergen solution was co-precipitated with tyrosine by the simultaneous addition of one volume of L-tyrosine in HCl (prepared by dissolving 24g L-tyrosine to 100ml with 3.4M HCl) and one volume of 3.2M NaOH, to four volumes of allergen solution, with vigorous agitation. The suspension so formed was centrifuged, washed repeatedly with buffered saline to remove contaminants and resuspended to the original volume in buffered saline pH6 ±1.

3-DMPL suitable for coadministration with the above formulation was prepared as follows.....

Biological activity

TH1 inducing activity in mice can be equated with the production of IgG2a and IgG2b antibodies and the TH2 inducing activity with the production of IgG1 antibodies and IgE antibodies.

Therefore, as an example, an experiment was carried out in mice to demonstrate the profiles of the allergen specific antibodies to an exemplar allergen ovalbumen (XOA) which is a well-known food allergen derived from chicken eggs. It was confirmed that a formulation consisting of MPL + XOA+tyrosine stimulated a more advantageous antibody profile than MPL + XOA, XOA+tyrosine or XOA alone.

Groups of mice were administered XOA (20ug) in various formulations:

1. XOA + tyrosine*
2. XOA+tyrosine +MPL*
3. XOA + MPL
4. XOA alone

*XOA was adsorbed onto tyrosine as described above for a pollen extract and then mixed with 3-DMPL (quantitiy?) immediately prior to administration.Antibodies specific for XOA were measured by an ELISA method

RESULTS

Formulation	IgG1 titre	IgG2a titre	IgG2b titre	IgE OD at 1/10 Dilut.
XOA+Tyrosine	102400	100	200	0.213
XOA+Tyrosine +MPL	409600	102400	102400	0.104
XOA + MPL	102400	200	400	0.218
XOA alone	6400	<100	<100	0.235
Normal Mouse Serum Values	<100	<100	<100	0.095

Of particular importance is the fact that the combination of allergen + tyrosine + MPL induces less allergen specific IgE antibody than the other combinations. Furthermore, the ratio of IgG2a or IgG2b to IgG1 antibodies is greater and consistent with the highest levels of the two former antibody isotypes seen in the experiment in the mice given allergen+tyrosine+MPL than in any other group of mice. This is indicative of a better ratio of TH1 cell induction over TH2 cell induction in this group compared with that induced in the other groups of mic